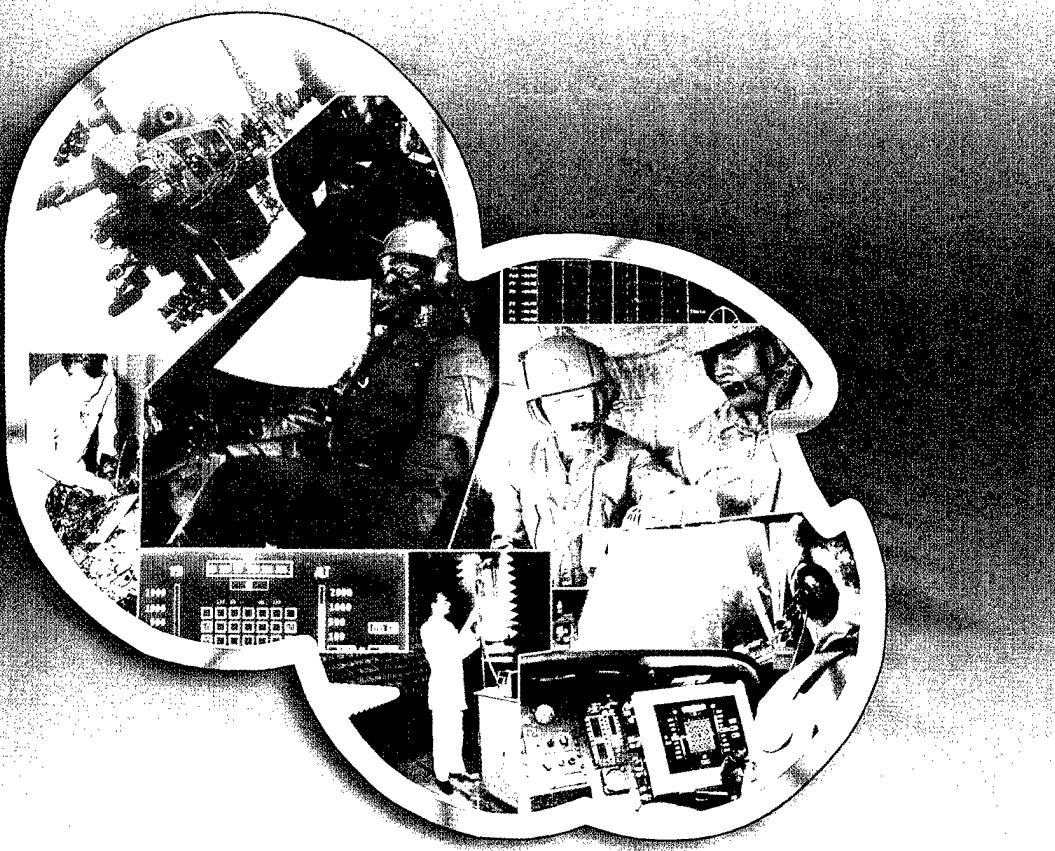


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# Dose Uniformity of Over-the-Counter Melatonin as Determined by High-Pressure Liquid Chromatography

by

Brian F. Prazinko, Joseph A. Sam, J. Lynn Caldwell and Alfred T. Townsend



Aircrew Health and Performance Division

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## Table of contents

	<u>Page</u>
Introduction.....	1
Methods.....	2
Materials.....	2
Procedure.....	2
Data analysis.....	3
Results.....	3
Discussion.....	4
References.....	7

## Appendix

Manufacturer's list.....	9
--------------------------	---

### List of tables

Values for means for each brand.....	5
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### List of figures

Means for each brand, respective lot and their standard deviations .....	6
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## Introduction

U.S. Army aviation personnel work at varying times in a 24-hour day. In addition, deployment across various time zones is common, thus requiring personnel to sleep and work at different times than they are accustomed. These changes in sleep/wake cycles lead to problems in obtaining adequate sleep, and thus, performance is compromised due to the lack of adequate rest. Although some people may be able to avoid working during times outside the "normal" alertness period, it is difficult to overcome the problems associated with sleeping outside the normal times.

One strategy that has been used by researchers in the past to help people adjust to working and sleeping outside the usual times is administration of melatonin (Arendt and Deacon, 1997; Dawson, Encel, and Lushington, 1995; Hughes and Badia, 1997; Knauth, 1997; Monk, 1986; Sack, Hughes, Edgar, and Lewy, 1997). While there is controversy over whether melatonin is successful as a hypnotic, there is considerable evidence that it can effectively alter circadian rhythms (Caldwell, 2000; Dawson and Armstrong, 1996; Lewy, Ahmed, Jackson, and Sack, 1992; Zaiden et al., 1994). Despite this, the clinical sleep community is concerned about melatonin's safety. There are many questions concerning melatonin's effects in pregnancy, its interaction with other medications, the impact of its long-term use, etc. (Arendt and Deacon, 1997). Not the least of these concerns is the quality control of over-the-counter (OTC) melatonin preparations (Czeisler and Turek, 1997; Sack, 1996; The National Sleep Foundation, 1997). Since melatonin can be sold as a dietary supplement, the Food and Drug Administration (FDA) has no control over the manufacturing of melatonin and the safety checks for impurities. In addition, the consistency of the stated dose may not be as stringent as those required by the FDA; for example, the actual melatonin content in the tablet compared to the stated dose may vary more than would be allowed by the FDA.

Army aviation personnel are potential users of melatonin, and confidence should be given that what is purchased in the store actually contains the amount of melatonin stated on the label. In order to determine whether OTC melatonin can be assumed to contain the labeled amount of melatonin which it purports, the melatonin made by several manufacturers was analyzed. If OTC melatonin contains the amount of melatonin which is claimed on the label, then it can be used with confidence. If the analyses indicate that some or all of the OTC melatonin preparations are not as the label claims, users can be educated in this regard.

## Methods

### Materials

Six tablets were analyzed from each of two separate lots of tablets made by eight different manufacturers (see appendix). High-pressure liquid chromatography (HPLC)-grade methanol (Fisher Scientific), melatonin, sodium acetate, ethylenediaminetetraacetic acid (EDTA) disodium salt, and acetic acid (Sigma) were all acquired from chemical supply companies.

### Procedure

Two bottles, each from a different lot of melatonin tablets (or capsules), were purchased at various health food and department stores representing eight manufacturers. Six tablets were drawn from each lot and analyzed to determine the melatonin content of each tablet.

An authentic 1 mM (millimolar) melatonin standard stock solution was prepared by dissolving 116.15 mg of N-acetyl-5-methoxytryptamine in 500 mL of HPLC grade methanol. A 3.0 mg/L standard was prepared by dilution of the stock solution with methanol. All standards were stored at 4°C.

Melatonin tablets (all of which were designated by manufacturer's label to contain 3 mg of melatonin) were finely powdered in a non-porous mortar and pestle (gel capsules were opened and the contained powder removed). Melatonin was extracted with 100 mL of methanol (vigorously shaken for 2 minutes). This extract was diluted to a concentration of one tablet per liter. Each sample (1.5 mL) was placed in a microcentrifuge tube (in duplicate), and centrifuged for 5 minutes at 3500 rpm to ensure sedimentation of suspended particles. All samples were stored at 2-8°C.

The procedure used to determine melatonin content in tablets was HPLC, which has been shown in the past to be sensitive to melatonin (Chin, 1990). A Bioanalytical Systems, Inc. (BAS) 200A HPLC instrument with unijet microbore C-18 column and electrochemical detector at 750 mV operated by BAS control software version 1.40 was used in conjunction with a Kontron 460 autosampler. A Neslab circulator maintained a sample temperature of 5°C. The mobile phase was similar to that of Laganà (1995) and consisted of 75% aqueous solution of 100 mM sodium acetate, 50 mM acetic acid, and 0.1 mM disodium EDTA (pH 4.9) and 25% acetonitrile. Using the autosampler, 10 µL injections were made. Melatonin peaks appeared at approximately 7 minutes with a flow rate of 0.05 mL/min at ambient temperature. Column pressure was approximately 1300 psi.

### Data analysis

The amount of melatonin per tablet was determined by a comparison of peak areas calculated with BAS report software version 1.4 set for an initial peak slope of 1.000 and a minimum area of 10,000 units. First and second runs for 6 tablets from each lot were injected immediately following a known standard, and peak areas for these 12 runs were compared to the preceding standard peak area. Data were gathered on a total of 16 lots (8 brands - 2 lots each), requiring 3 separate test sessions to complete all runs. For each of the three test sessions, all standards were averaged and analyzed to determine variability of the method. The calculated melatonin content of each tablet was compared with the z-score calculated from the average and standard deviation of all the tablets.

### Results

All standards were made to be precisely 3 mg/L. The variability of peak areas resulted in a value of  $3.000 \pm 0.150$  for 12 runs in test session one,  $3.000 \pm 0.123$  for 6 runs in test session two, and  $3.000 \pm 0.288$  for 8 runs in test session three. The overall value for the 26 standard runs was  $3.000 \pm 0.198$  mg/L. The 96 tested tablets had an average value of  $3.091 \pm 0.460$  mg. For 2 of the 96 tablets, only 1 of the 2 runs was completed as a result of equipment synchronization difficulty.

The table shows each brand and their respective tablets' melatonin content averaged from two runs, as well as the tablet's z-score compared to all measured tablets and the lot average (within each brand) with standard deviation for all tablets within that lot. Of the tablets analyzed (across all brands and lots), 12 tablets had z-scores greater than 1 standard deviation above the tablet mean, and 15 tablets had z-scores greater than 1 standard deviation below the tablet mean. One lot within one brand across all 6 tablets had z-scores greater than one standard deviation both above and below the tablet mean, while two lots had no z-scores greater than one standard deviation either above or below the tablet average.

The figure shows average melatonin content for each lot with standard deviation. The solid line represents the overall tablet average and the dotted line represents their standard deviation.

### Discussion

This brief study indicates that questions concerning the actual melatonin content of OTC preparations may be justified. When the standard deviation for the tablets is compared to the standard deviation of the procedure standard, it is clear that the 2.3 fold larger standard deviation for the tablets indicates variability in tablet melatonin content which can be attributed to the tablets rather than to the HPLC procedure. While the methods used in this inquiry could result in individual discrepancies, the great difference in variance between the authentic melatonin runs and the tablet runs likely points to differences in actual tablet melatonin content.

These results indicate that users of OTC melatonin should be aware that the variability in doses for individual tablets is high, and they may be taking more or less melatonin than is stated on the package label. Whether this is a health concern or not has yet to be determined. It may not be significant if one consumes 2 mg or 4 mg of melatonin rather than the expected 3 mg. The safety issues of using melatonin have yet to be determined, but it appears from the literature that a small discrepancy in dosage from what is expected may not be a problem. However, further study is needed to determine whether other concerns are warranted about the manufacturing of melatonin. While the dose uniformity of OTC melatonin is a concern, the purity of the tablets (what else may be in the tablet due to manufacturing laxity), as well as other formulation issues that may affect its absorption into the bloodstream should also be studied. Once these variables are taken into account, consumers can be educated about the quality of OTC melatonin.

Table.

Values for means for each brand. Z-scores higher than 1 or lower than -1 are highlighted.

Brand	Lot	Tablet 1	Tablet 2	Tablet 3	Tablet 4	Tablet 5	Tablet 6	Mean	SD
1	1	4.183029	3.380429	3.370829	3.026770	3.253036	3.415427	3.438253	0.391593
	Z	2.375668	0.629853	0.608971	-0.13943	0.352747	0.705981		
	2	3.680631	3.781942	3.438091	3.349509	3.021503	3.038436	3.385019	0.316631
	Z	1.282851	1.503224	0.75528	0.562596	-0.15088	-0.11405		
2	1	3.364672	3.327781	3.316267	3.94914	3.303347	3.171938	3.405524	0.274299
	Z	0.595578	0.515333	0.490288	1.866913	0.462185	0.176344		
	2	2.829457	3.330146	3.198975	3.046241	3.568508	2.929076	3.150400	0.272881
	Z	-0.56862	0.520476	0.235155	-0.09707	1.038962	-0.35193		
3	1	2.834334	2.569549	2.757312	2.723407	2.493965	2.648665	2.671205	0.125703
	Z	-0.55801	-1.13397	-0.72555	-0.79930	-1.29838	-0.96188		
	2	4.189923	3.008142	2.802477	3.052682	3.084579	3.326297	3.244016	0.492774
	Z	2.390663	-0.17995	-0.62731	-0.08306	-0.01368	0.512104		
4	1	3.019919	2.681001	2.707624	2.683022	3.097049	3.195714	2.897388	0.233522
	Z	-0.15433	-0.89154	-0.83363	-0.88715	0.013444	0.228061		
	2	3.602439	3.053351	3.277206	3.244723	3.128686	3.104085	3.235082	0.199161
	Z	1.112768	-0.08161	0.405322	0.334666	0.082261	0.028749		
5	1	2.555349	2.600028	2.580022	2.662594	2.758895	2.700966	2.642976	0.078321
	Z	-1.16486	-1.06767	-1.11119	-0.93158	-0.72211	-0.84811		
	2	2.798595	2.570106	2.970367	2.526545	2.946798	3.119878	2.822048	0.235631
	Z	-0.63575	-1.13276	-0.26211	-1.22751	-0.31338	0.063102		
6	1	2.833228	3.290963	3.388253	3.653859	3.384549	3.468144	3.336499	0.275096
	Z	-0.56042	0.435246	0.646872	1.224617	0.638815	0.820651		
	2	3.498522	3.353296	2.805245	3.541155	3.485759	3.269124	3.325517	0.274497
	Z	0.886729	0.570834	-0.62129	0.979465	0.858967	0.387742		
7	1	3.044974	2.496905	2.679193	3.179384	2.291380	2.420076	2.685319	0.356239
	Z	-0.09983	-1.29199	-0.89548	0.192541	-1.73905	-1.45911		
	2	2.433669	2.513502	2.569159	2.937264	2.469229	2.889040	2.635311	0.220459
	Z	-1.42954	-1.25589	-1.13482	-0.33412	-1.35219	-0.43902		
8	1	3.054744	4.298784	2.632921	2.673185	2.726657	2.149340	2.922605	0.734001
	Z	-0.07858	2.627457	-0.99612	-0.90854	-0.79223	-2.04801		
	2	3.321960	3.035843	4.173585	4.250118	3.091534	4.067289	3.656722	0.566538
	Z	0.502671	-0.11969	2.355126	2.521600	0.001448	2.123911		

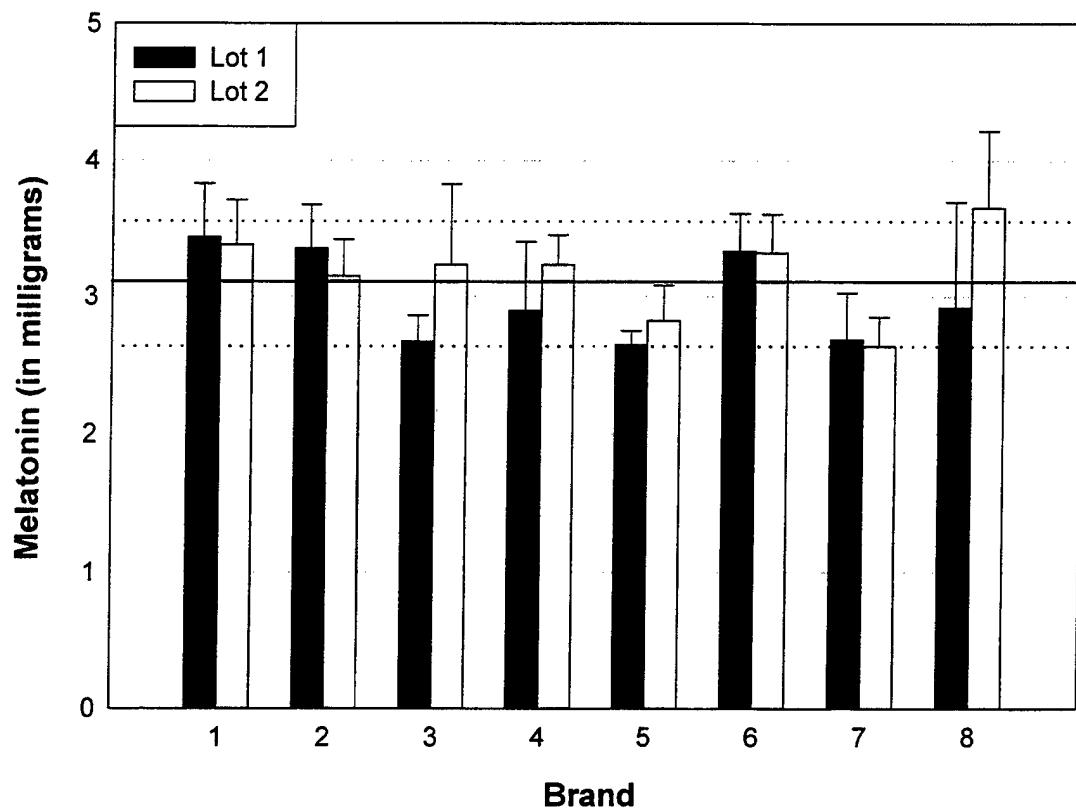


Figure. Means for each brand, respective lot and their standard deviations. The solid line represents the pill grand mean and the dotted lines represent its average standard deviation.

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Appendix.

Manufacturers list.

Bioanalytical Systems (BAS)  
2701 Kent Avenue  
West Lafayette, IN 47906  
USA

Fisher Scientific  
3970 John's Creek Ct., Ste. 500  
Suwanee, GA 30024  
USA

General Nutrition Corporation  
300 Sixth Avenue  
Pittsburgh, PA 15222  
USA

IBSD Corporation  
P.O. Box 8233  
Dothan, AL 36304  
USA

Kontron Instruments Limited  
Blackmoor Lane  
Crowley Business Park  
Watford, WD1 8XQ  
England

Neslab Instruments  
P.O. Box 1178  
Portsmouth NH 03802-1178  
USA

Nature-Made Nutritional Products  
15451 San Fernando Mission Boulevard  
Sun Valley, CA 91346  
USA

Natrol Incorporated  
21411 Prairie Street  
Chatsworth, CA 91311  
USA

Now Foods  
395 South Glen Ellyn Road  
Bloomingdale, IL 60108  
USA

Nutraceutical International  
1400 Kearns Boulevard Floor 2  
Park City, UT 84068  
USA

Schiff Products, Incorporated  
1960 South 4520 West  
Salt Lake City, UT 84104  
USA

Sigma  
P.O. Box 14508  
St. Louis, MO 63178  
USA

Twin Lab Incorporated  
2120 Smithtown Avenue  
Ronkonkoma, NY 11779  
USA